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14. ABSTRACT
Previously, mapping of the 9p23-24 amplicon in esophageal cancer cell lines led us to the positional cloning of GASC1which encodes a histone demethylase. However, the transforming roles of GASC1 in breast cancer remain to be determined. In this study, we identified GASC1 as one of the amplified genes for the 9p23-24 region in breast cancer, particularly in basal-like subtypes. Our in vitro assays demonstrated that increased expression ofGASC1 induces transformed phenotypes, including growth factor-independent proliferation, anchorage-independent growth, altered morphogenesis in Matrigel, and mammosphere forming ability, when over expressed in immortalized, nontransformed mammary epithelial MCF10A cells. Additionally, GASC1 demethylase activity may be linked to the stem cell phenotypes in breast cancer. Thus, GASC1 is a driving oncogene in the 9p23-24 amplicon in human breast cancer and targeted inhibition of GASC1 histone demethylase in cancer could provide potential new avenues for therapeutic development.

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Introduction

The *GASC1* gene encodes a histone demethylase and regulates epigenetic histone modifications, which has a central role in maintaining gene expression programs that are important for stem cell self-renewal and differentiation. It is located in the core region of the 9p23-24 amplicon that is frequently observed in basal-like breast cancer cells. Basal-like breast cancer is a poorly differentiated aggressive cancer subtype and bears an embryonic stem cell-like gene expression signature. We hypothesize that GASC1 is the driving oncogene behind transformation and acquisition of cancer stem cell phenotypes in basal-like breast cancer cells. Furthermore, because basal-like cancer cells have intrinsic drug-resistance against traditional chemotherapy, we propose GASC1 as a potential therapeutic target in the treatment of breast cancers, particularly of the basal-like subtype.

Body

1, Specific Aims

The specific aims of this project are:
(1) To investigate the molecular mechanism, including the structural details, of GASC1 that are involved in their transforming and stem cell phenotypes in basal-like breast cancer cells.
(2) To examine the potential of GASC1 as a therapeutic target in aggressive, basal-like breast cancer.

2, Studies and Results

Task 1. To investigate the molecular mechanism, including the structural details, of GASC1 that are involved in their transforming and stem cell phenotypes in basal-like breast cancer cells.

GASC1, also known as *JMJD2C/KDM4C*, was originally discovered in a KYSE150 cell line established from a poorly differentiated esophageal squamous cell carcinoma. Recently, *GASC1* gene amplification and over expression have been demonstrated in various tumor types, including sarcomatoid carcinoma of the lung, mucosa-associated lymphoid tissue lymphoma, medulloblastoma , prostate and breast cancer. More recent, our laboratory, together with others, demonstrated that GASC1 amplification and over expression is more prevalent in the basal-like breast cancer. The basal-like breast cancers, which account for about 25% to 30% of all tumors, are among the most aggressive forms of breast cancer. Further, an embryonic stem (ES)-like gene expression signature is associated with high-grade ER-negative tumors, often of the basal-like breast cancer.

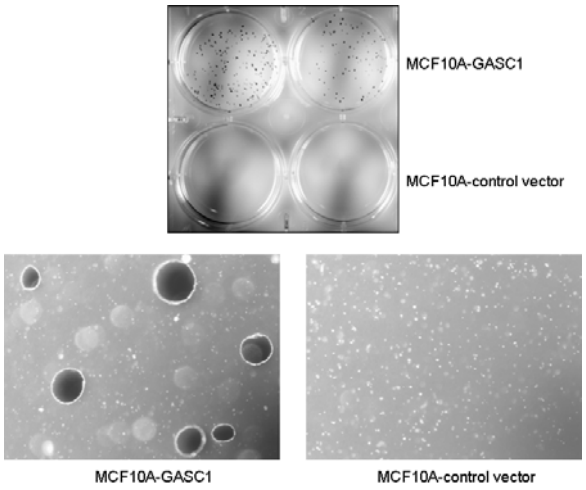

Figure 1. Representative pictures of MCF10A-GASC1 and control cell soft-agar colonies. Cells were grown for 4 weeks in soft agar and stained with the vital dye p-iodonitrotetrazolium violet.

To address the question of whether GASC1 possesses transforming properties, we first used the pLentiviral expression construct of wild-type GASC1 to establish the MCF10A-GASC1 cell line derived from the human non-transformed mammary epithelial MCF-10A cells. Over-expression of GASC1 mRNA and protein in this cell line was confirmed with semiquantitative RT-PCR and western blot, respectively. To avoid clonal selection effects, all experiments were performed with drug-selected pools of cells that stably over-express GASC1. Over expression of GASC1 in MCF10A cells resulted in phenotypic alterations that are hallmarks of neoplastic transformation, including growth factor-independent proliferation and anchorage-independent growth in soft agar (Figure 1). Further, three dimensional morphogenesis in Matrigel indicated that GASC1 over expression disrupts epithelial cell architecture, which occurs frequently during the early stages of cancer formation.

To determine if eleveated expression of the GASC1 gene could induce phenotypes of cancer stem cells, we performed mammosphere formation assays with MCF10A and MCF10A-GASC1 cells. As shown in Figure 2, MCF10A-GASC1 cells have more than 5-fold higher capacity to generate mammospheres than MCF10A control cells after 10-12 days in the mammosphere cultures. When the

cells from mammospheres were re-plated under normal culture conditions, viable cells in the mammospheres adhered to the culture dish and proliferated; resulting in a large number of proliferative colonies derived from MCF-10-GASC1 cells compared to parental MCF-10A cells.

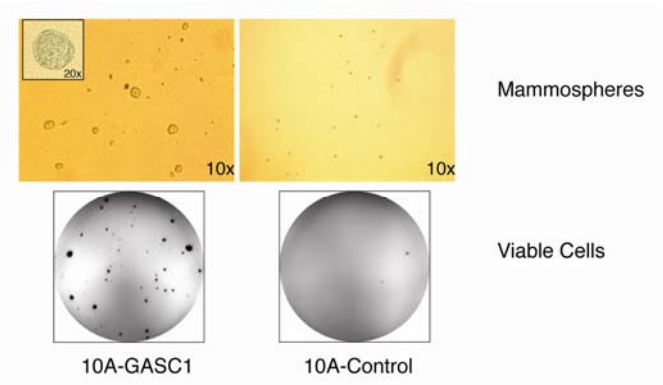

Figure 2. Mammosphere formation assay of MCF10A-GASC1 and MCF10A control cells. The top- panel shows representative images of mammospheres formed from MCF10A-GASC1 cells and MCF10A control cells on day 12. The bottom panel shows viable cells in the mammospheres adhered to the culture dish after replanting mammosphere culture into the attachment plate.

GASC1 has multiple functional domains, including a demethylase catalytic domain and lysine binding domains. We successful cloned a series of GASC1 pLentiviral constructs that delete or mutate the enzymatic domains of the GASC1 gene and stably over expressed these mutants in MCF10A. Our *in vitro* assays demonstrated that over-expressing of wild-type GASC1 protein, but not that of GASC1 mutant proteins with defective enzymatic domain, renders MCF-10A cells growth factor independent proliferation. This piece of preliminary data indicates that demethylase activity of GASC1 is required for its oncogenic activity.

***Task 2*. To examine the potential of GASC1 as a therapeutic target in aggressive, basal-like breast cancer.**

To further assess endogenous GASC1's involvement in the transformation of breast cancer cells and to establish proof-of-concept of targeted treatment, we knocked down GASC1 expression and examined the effect on proliferation of breast cancer cells with GASC1 gene amplification, and control MCF10A cells, which does not have GASC1 gene amplification. We used an Expression Arrest GIPZ lentiviral shRNAmir system from OpenBiosystems (http://www.openbiosystems.com/) to stably knock down GASC1 expression. In this pGIPZ vector, TurboGFP and shRNA are part of a single transcript allowing the visual marking of the shRNA expressing cells. HCC1954 and Colo824 cells with high-level GASC1 gene amplification were infected with the lentivirus supernatants for knock-down of GASC1. Non-silencing shRNA lentiviral control, at the same titer as GASC1 shRNA, was used in parallel as the negative control shRNA.

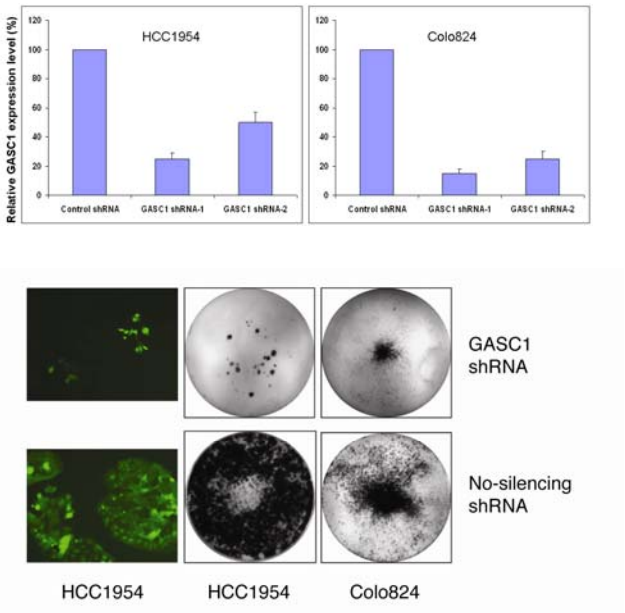

Figure 2. Top: shRNA knock down GASC1 expression in HCC1954 and Colo824 breast cells (1= pLKO-shRNA, 2= pGIPZ-shRNA). Bottom: shRNA-mediated knock down of GASC1 inhibits colony formation in breast cancer cells with GASC1 amplification. The left panels showed the TurboGFP fluorescence of pGIPZ shRNA in HCC1954 cells after 2 weeks.

Forty-eight hours after infection, cells were selected with complete growth medium containing puromycin. Pooled cell clones were monitored for TurboGFP expression by fluorencence microscopy and GASC1 mRNA expression levels were measured by semiquantitative RT-PCR. Semiquantitative RT-PCR experiments revealed that the GASC1-shRNA cell clones showed down-regulation of GASC1 expression to 20-50% of the level seen in the non-silencing infected cell clones (Figure 3). Next, the consequences of decreased GASC1 expression on colony formation were evaluated. Figure 2 shows that GASC1 knock down dramatically slowed cell growth and inhibited colony formation of HCC1954 and Colo 824 cells. The dramatic inhibition of HCC1954 and Colo824 cell growth by knock-down of GASC1 were reproduced with a pLKO GASC1 shRNA. The knock-down of GASC1 inhibited cell growth of SUM-149 by ~ 50% and had only a slight effect on the growth of MCF10A cells. Taken together, these results are consistent with a transforming function for GASC1 when it is over-expressed in human breast cancer.

Remaining work for no-cost extension: more growth phenotype tests such as *in vitro* mammosphere assays; detect expression of the mammary gland cell-lineage-specific markers; measure specific stem cell markers in the GASC1 knock-down breast cancer cells; and test GASC1-specific small molecular inhibitors in basal breast cancer cells.

Key Research Accomplishments

In this study, we report that over expression of GASC1 in human nontransformed mammary epithelial cells results in phenotypic alterations that are hallmarks of neoplastic transformation, including growth factor-independent proliferation and anchorage-independent growth in soft agar. Additionally, GASC1 demethylase may be linked to the stem cell phenotypes in breast cancer. Thus targeted inhibition of GASC1 histone demethylase in cancer could provide potential new avenues for therapeutic development for basal breast cancer.

Reportable Outcomes

Abstracts:

1. Yang Z-Q. Genomic amplification and oncogenic properties of the GASC1 histone demethylase gene in breast cancers. Eighth Joint Conference of AACR-JCA: Cancer Genomics, Epigenomics, and the Development of Novel Therapeutics, Hawaii, HI, February 5-9, 2010.
2. Yang Z-Q. histone demethylase GASC1 in cancer. BIT's 3rd World Cancer Congress 2010, Singapore, June 22-25, 2010

Conclusion

Previously, mapping of the 9p23-24 amplicon in esophageal cancer cell lines led us to the positional cloning of *GASC1 (gene amplified in squamous cell carcinoma 1)*, which encodes a nuclear protein with a Jumonji C (JmjC) domain that catalyzes lysine (K) demethylation of histones. However, the transforming roles of GASC1 in breast cancer remain to be determined. In this study, we identified *GASC1* as one of the amplified genes for the 9p23-24 region in breast cancer, particularly in basal-like subtypes. The levels of GASC1 mRNA expression were significantly higher in aggressive, basal-like breast cancers compared with non basal-like breast cancers. Our *in vitro* assays demonstrated that increased expression of GASC1 induces transformed phenotypes, including growth factor-independent proliferation, anchorage-independent growth, altered morphogenesis in Matrigel, and mammosphere forming ability, when over expressed in immortalized, nontransformed mammary epithelial MCF10A cells. Additionally, GASC1 demethylase activity may be linked to the stem cell phenotypes in breast cancer. Thus, *GASC1* is a driving oncogene in the 9p23-24 amplicon in human breast cancer and targeted inhibition of GASC1 histone demethylase in cancer could provide potential new avenues for therapeutic development.